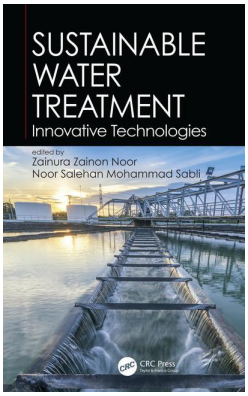

Sustainable Water Treatment Innovative Technologies

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Optimization of Lipid Content in Microalgae Biomass Using Diluted Palm Oil Mill Effluent by Varying Nutrient Ration

Publication details
https://test.routledgehandbooks.com/doi/10.1201/9781315116792-3
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Published online on: 18 Apr 2017

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Noor Amirah Abdul Aziz, Shreeshivadasan Chelliapan, Mohanadoss Ponraj, Mohd Badruddin Mohd Yusof, and Mohd. Fadhil Md Din

CONTENTS

3.1 Introduction

The oil palm is one of the world's most rapidly expanding equatorial crops. In Malaysia, oil palm plantation currently occupies the largest acreage of farmed land and the palm oil industry is growing rapidly. Malaysia is one of the major palm oil producers in the world (Lam et al., 2009). While the palm oil industry has been recognized strongly for its contribution toward economic growth and rapid development, it has also contributed to environmental pollution due to the production of large quantities of by-products during the process of oil extraction (Parthasarathy et al., 2016). Microalgal culture has received more attention, given its prospects as a source of bioenergy and its potential for wastewater treatment. In this respect, simple and easily cultivated biomass has a number of applications, ranging from its direct use such as biodiesel and various pigments (Fulton, 2004). The complication of cultivation methods and the high cost of growth medium have become a major drawback for the algal industry; nevertheless, the integration along with wastewater treatment has provided a feasible solution due to the fact that exploitation of wastewater as the source of growth medium simultaneously eliminates the requirement for an expensive medium and at also remediates the wastewater.

The current study investigates the potential, benefits, strategies, and challenges of microalgae to be integrated with wastewater treatment, particularly palm oil mill effluent (POME) treatment in Malaysia, which due to the hazardous properties of POME may lead to severe environmental pollution. The integration of POME treatment using microalgal culture will potentially reduce the wastewater treatment retention time and eliminate toxic elements, which serve as nutrients for the growth of microalgae. Moreover, microalgae are gaining considerable attention as a feedstock for lipid production as they can be grown away from the croplands and hence do not compromise food crop supplies (Liu et al., 2008). The optimization of lipid content in microalgal biomass using diluted POME by changing the nutrient ratio is discussed in this study.

3.1.1 Background of Study

The Malaysian palm oil industry is growing rapidly and becoming a very important agriculture-based industry, where the country today is the world's leading producer and exporter of palm oil, replacing Nigeria as the chief producer since 1971. As the world's largest palm oil producer, Malaysia produced 10.6 million tonnes of palm oil in 1999 and increased to 17.7 million tonnes of palm oil in 2008 (MPOB, 2010). This figure is expected to rise as the demand for palm oil increases since it is one of the most important vegetable oils in the world's oil and fats market. The total oil palm planted area in the country in the year 2009 alone was 4.69 million hectares.

Unfortunately, this vital agricultural and industry activity generates a significant amount of by-product known as POME. For every ton of product produced in the extraction process, about 2.5–3.5 tonnes of POME are generated. The effluent is nontoxic as no chemicals are added into the extraction process, and it is fairly acidic with pH ranging from 4.0 to 5.0 as it contains organic acids in complex forms that are suitable to be used as carbon sources (Wu et al., 2010). If the discharged effluent is not properly treated, it can surely cause substantial environmental problems. The palm oil mill industry in Malaysia is identified as the one generating the largest pollution load in rivers throughout the country.

Algae are commonly found in the water system. Algae can be used for wastewater treatment to eliminate organic carbon from wastewater systems. Algae can be categorized into two main groups; microalgae and macroalgae. Microalgae can grow under two conditions: autotrophic and heterotrophic. Autotrophic microalgae are the producers in a food chain, such as plants on land or algae in water. Heterotrophic microalgae are organisms that use organic carbon for growth. A microalga is a photosynthetic microorganism that is able to use solar energy to combine water with carbon dioxide to create biomass (Widjaja et al., 2009). Microalgae have been suggested as a very suitable candidate for fuel production because of their advantages of higher photosynthetic efficiency, higher biomass production, and faster growth compared to other energy crops. Many microalgae strains have been identified capable of producing high content of lipid and most of them are marine microalgae. POME is a carbon source for microalgae that will help the growth of microalgae. Different organic loading will cause different growth of microalgae.

3.1.2 Problem Statement

The palm oil industry is among the main production industries in Malaysia. The palm oil industry is identified as the one generating the largest pollution load in rivers throughout the country. With a thick brownish colloidal mixture of water, oil, and suspended solids, it possesses a very high BOD_3 which is a hundred times more polluting than domestic waste and this adversely affects the water environment. A suitable treatment needs to be worked out since POME has higher potential toward the production of biofuel. This effluent can be used as one of the biofuel resources using microalgae to produce biomass in producing lipid to produce biodiesel. The abundance of POME in Malaysia is an advantage in producing this biofuel.

3.1.3 Scope of Study

This study was conducted to observe the ability of *Chlorella pyrenoidosa* and *Chlorella vulgaris* in diluted POME with concentration of 250 mg/L in different concentrations of nutrient, in terms of lipid production. The microalgae

were cultured in Bold's Basal Medium (BBM) for 2 weeks for the acclimatization process before conducting tests. The focus of this study is microalgae produced higher lipid content in POME culture.

3.1.4 Significance of Study

There are a lot of components in the POME substance, that could be useful especially in producing biofuel. Biodiesel is a future product that would be important due to its ability to be used as an alternative fuel. Besides, biodiesel has biodegradable behavior and it is a renewable energy.

3.2 Literature Review

3.2.1 Microalgae

Microalgae known as microphytes are a microscopic algae, which can be found in freshwater and marine systems. They are unicellular cells, which can exist individually, in groups or chains. Mata et al. (2010) have stated that microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure.

Microalgae do not look like higher plants. They do not have roots, stems, or leaves but are capable of performing photosynthesis. They use carbon dioxide gas as the food source to grow photoautotrophically.

Mata et al. (2010) stated that microalgae can provide feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol, and bioelectricity. Algae biodiesel contains no sulfur and performs as well as petroleum diesel, while reducing emissions of particulate matter, CO, hydrocarbons, and SO_x. NO_x may be higher in some engine types.

Microalgae have been suggested as a very suitable candidate for fuel production because of their advantages of higher photosynthetic efficiency, higher biomass production, and faster growth compared to other energy crops (Widjaja et al., 2009).

3.2.1.1 Cell Nutrients

For cell protoplasm, algae need a source of carbon other than light. Carbon dioxide is the primary carbon source for algae. Algae grow much better in waters containing high concentrations of bicarbonate alkalinity than in waters with low bicarbonate alkalinity. For algae protoplasm, nitrogen is necessary to form proteins. Ammoniacal nitrogen is the primary source of nitrogen for algae with nitrates as the secondary source. Nitrates must be reduced to ammonia

for incorporation into protoplasm. Phosphorus is a critical element for the growth of algae. Although algae do not need a large quantity of phosphorus, it is important in energy transfer for the algae. Phosphates are the primary source of phosphorus for the algae. Since phosphates are limited in the natural environment, phosphorus availability is often the limiting factor in the growth of algae. Algae also need sulfates, trace metals, iron, and magnesium.

3.2.1.2 Growth

The growth of algae follows the same general pattern as the growth of bacteria and fungi. In an excess of light and nutrients, growth is restricted only to the ability of the algae to process the nutrients. Unrestricted growth results in a log increase of cell mass. Algae undergo endogenous respiration. In the presence of light, the nutrients released by endogenous respiration are immediately metabolized back to normal protoplasm. The rate of endogenous respiration is directly proportional to the active mass of algae. Proteins are the primary materials undergoing endogenous respiration and leaving cell wall polysaccharides.

3.2.2 Heterotrophic and Autotrophic Conditions

An organism is heterotrophic when it uses organic carbon for growth. On the other hand, an autotroph organism uses a source of energy such as light to produce organic substrate from inorganic carbon dioxide.

3.2.3 Biodiesel

Biodiesel consists of fatty acid methyl esters originating from vegetable oils and animal fats (Widjaja et al., 2009; Feng et al., 2011). It is a biodegradable, renewable, and nontoxic fuel. Furthermore, it contributes no net carbon dioxide or sulfur to the atmosphere and emits less gaseous pollutants than normal diesel (Widjaja et al., 2009). Biodiesel industries are expanding rapidly both in the United States and Europe with soybean or rapeseed oils as the feedstock (Feng et al., 2011).

Biodiesel is a mixture of fatty acid alkyl esters obtained by transesterification of vegetable oils or animal fats. For biodiesel production, lipids and fatty acids have to be extracted from the microalgal biomass. The most common ways are transesterification as the biodiesel from transesterification can be used directly or as blends with diesel fuel in diesel engines. Biodiesel primarily rapeseed methyl ester, has been in commercial use as an alternative fuel since 1988 in many European countries (Lang et al., 2001).

In particular, biodiesel has two main advantages. First, biodiesel helps in terms of mitigation of the excessive emission of carbon dioxide. Second, biodiesel can be a substitute for petroleum. The key processes involved in biodiesel production using microalgae are cultivation, harvest, lipid extraction (cell disruption), and the transesterification of the lipid (Lee et al., 2010).

3.2.4 Palm Oil Mill Effluent

POME is a waste produced from palm oil processing plants. This oily waste is produced in large volume, and it needs an efficient treatment to avoid environmental hazards. The palm oil industry in Malaysia is growing rapidly and is among the major production industries in Malaysia. Malaysia is one of the world's leading producers and exporters of palm oil. Large quantities of water are used during the extraction process of crude palm oil from the fresh fruit. About 50% of the water results in POME. It is estimated that for 1 tonne of crude palm oil produced, 5–7.5 tonnes of water will end up as POME. In the year 2004, more than 40 million tonnes of POME were generated from more than 370 mills in Malaysia, and this amount keeps increasing. In the year 2008 alone, at least 44 million tonnes of POME were generated from 410 operated mills (Wu et al., 2010). Thian et al. (2010) found that microalgae can be found in POME. Typically with very high organic content and oil, the resulting fresh POME is a thick brownish color liquid. In addition, this type of POME is discharged at a high temperature, between 80°C and 90°C. It possesses a high BOD_3 which is about a hundred times more polluting than domestic waste.

The characteristics of POME may vary considerably for different batches, days, and factories, depending on the processing techniques and the age or type of fruit as well as the discharge limit of the factory, climate, and condition of the palm oil processing (Wu et al., 2010).

3.2.4.1 Treatment of POME

Ponding is the most commonly used system in Malaysia to treat POME. A typical ponding system is as follows: raw POME generated from the milling processes is discharged into the raw pond. Adequate hydraulic retention time (HRT) is provided for the settling process to take place in the raw pond. The raw POME is flowed into an anaerobic pond in which anaerobic degradation occurs, followed by an aeration pond, and then discharged to a watercourse (Neoh et al., 2015).

3.2.4.1.1 Aerobic Digestion

A system using an aerobic digestion for POME treatment would be more efficient and HRT is even shorter than an anaerobic system. Many researchers have found that using an aerobic system, the COD removal in the waste is high; more than 95% can be achieved in a much shorter HRTs than in usual anaerobic digestion.

3.2.4.1.2 Anaerobic Digestion

Anaerobic digestion is the most suitable method for the treatment of effluents containing a high concentration of organic carbon such as POME. The suggested anaerobic treatment process for POME includes the anaerobic

suspended growth process, attached growth anaerobic process, anaerobic sludge blanket process, membrane separation anaerobic treatment process, and hybrid anaerobic treatment process. Today, 85% of POME treatment is based on an anaerobic and facultative ponding system, which is followed by another system consisting of an open tank digester coupled with extended aeration in a pond (Vijayaraghavan et al., 2007).

3.2.4.1.3 Bioreactor System

This is a simple and innovative bioreactor process that is capable of treating POME efficiently. The system is superior to the conventional system as it operates with a very short HRT, takes high organic loading, requires less space, and is more environmentally friendly.

3.3 Methodology

Figure 3.1 will shows the methodology of research.

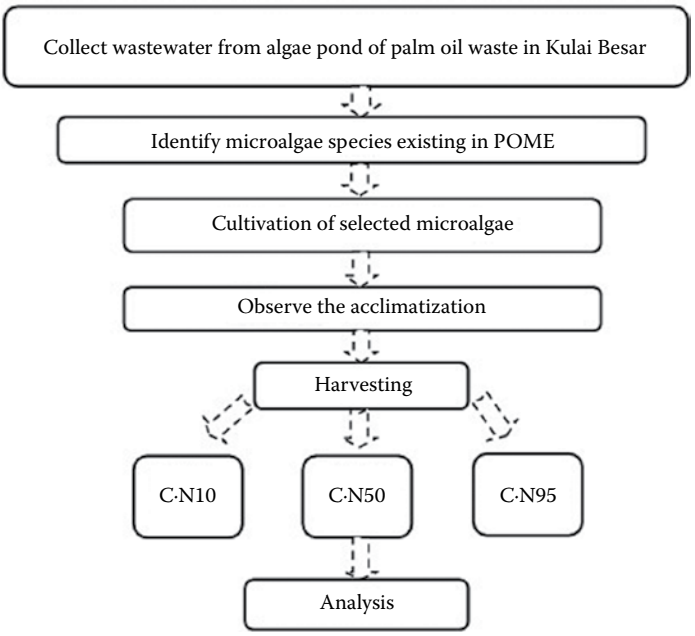

FIGURE 3.1
Research framework.

3.4 Results and Analysis

3.4.1 Identification of Microalgae

A light microscope aided in identifying microalgae species in the culture samples. Slides with microalgae were then observed under the microscope with 10 times magnification. Microalgae existing in POME wastewater were identified. Wastewater for the experiment was collected from an algae pond of palm oil waste in Felda Kulai Besar, Johor.

The observed algae found will then be referred to the nearest morphology described by Bersanti and Gualtieri (2006) in algae dichotomous key identification. The sample most probably consists mainly of *Chlorella pyrenoidosa* as well as *Spirogyra* sp. According to Bersanti and Gualtieri (2006), most of the microalgae sizes observed were to be around 1 μm to a few millimeters only.

Chlorella pyrenoidosa was found to be dominant over *Spirogyra* sp. Adaptation to nutrients from POME has decided the types of dominant microalgae species in a community. The high concentration of organic nutrient available in POME is more favorable for *Chlorella pyrenoidosa* hence more dominant over other species present in the sample. The microalgae found in POME are shown in Figure 3.2a–d.

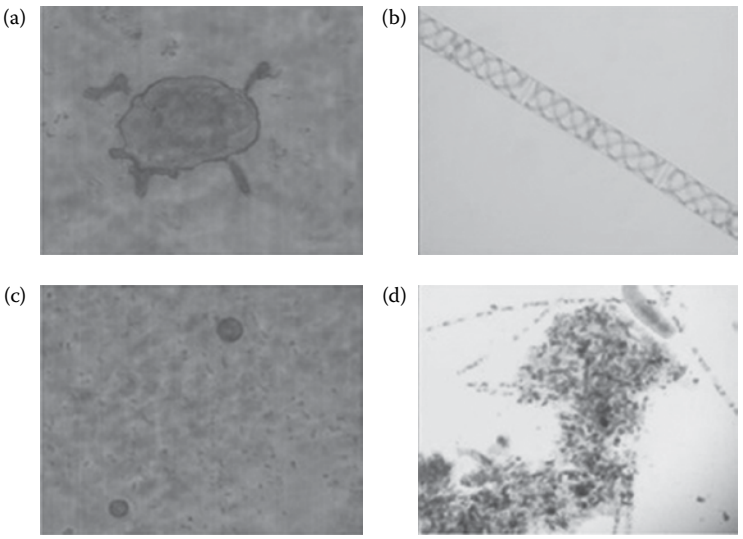

FIGURE 3.2
The microalgae found in POME. (a) *Chlorella*. (b) *Spirulina*. (c) Microalgae cells 10× dilution. (d) Microalgae cells 10× dilution.

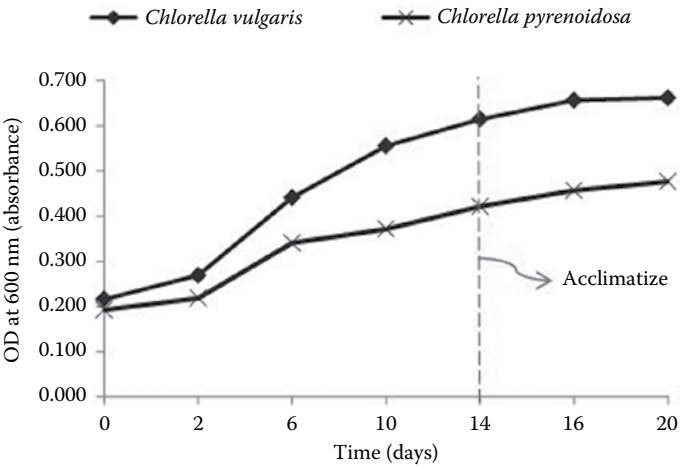

FIGURE 3.3
Chlorella vulgaris and *Chlorella pyrenoidosa* acclimatization.

3.4.2 Acclimatization of Microalgae

Since *Chlorella vulgaris* comes in a synthetic medium, it is of interest to know whether the chlorella can be adapted to POME conditions or not. From the findings, it shows that *Chlorella vulgaris* and *Chlorella pyrenoidosa* can adapt to POME wastewater. As we know, *Chlorella pyrenoidosa* exists in POME, but from the findings, it shows that *Chlorella vulgaris* can grow better than *Chlorella pyrenoidosa*. Figure 3.3 shows at day 10, both the microalgae start to stabilize, and the acclimatization is almost constant.

3.4.3 Determination of Carbon/Nitrogen Ratio (C/N)

A total nitrogen test and COD test was conducted to investigate the carbon/nitrogen ratio. The findings are as shown in Table 3.1. C/N ratio was

TABLE 3.1
Carbon/Nitrogen Composition in Various Ratios

Raw Pome: Algae Pond	COD Total	Total Nitrogen	C/N Ratio
10:90	2275	313	100:14
50:50	32,800	3010	100:9
70:30	27,200	2300	100:8.5
80:20	39,000	4340	100:11
90:10	44,900	3540	100:8
95: 5	57,400	3530	100:6

determined in 250 mg/L concentration of POME in 1 L medium. Using the equation $m_1v_1 = m_2v_2$ the C/N ratio was obtained. The experiment was carried out in 10 mL of raw POME and algae pond mixing. 10:90 ratio indicates that, in 10 mL of the mixing, 1 mL is raw POME and 9 mL is algae pond. Table 3.1 shows that the existence of a lot of raw POME in the solution will obtain lower C/N ratio value while having a limited amount of raw POME, a high concentration of nutrient ratio will be obtained. To determine which variable will be used in the next experiment, higher, middle, and lower C/N ratios are chosen to investigate which concentration of C/N ratio will give higher lipid content. Feng et al. (2011) state that depletion in nutrient ratio will produce higher lipid content and higher nutrient ratio will produce lower lipid content.

3.4.4 Growth Rate of Microalgae

The optical density (OD) test, and mixed liquor suspended solid (MLSS) test were performed to determine the growth rate of *Chlorella pyrenoidosa* and *Chlorella vulgaris*. The variation of the substrate ratio in OD with time for 20 days of batch operation is depicted in Figure 3.4a and b. Both the strains of microalgae achieve optimum growth at 95:05 ratio. The microalgae could not appropriately grow at a 10:90 ratio. It shows a similar trend with MLSS behavior (Figure 3.5a and b).This means that both of the microalgae would be suitable at 95:05 ratio for growth.

3.4.5 Cell Biomass

Cell biomass determination was conducted through chemical and physical tests. For chemical observation, the COD biomass test was conducted, while for the physical test the cell dry weight test was done. Both strains show similar trends in COD biomass and cell dry weight but *Chlorella pyrenoidosa* at ratio 10:90, shows different results. This is due to the human error done

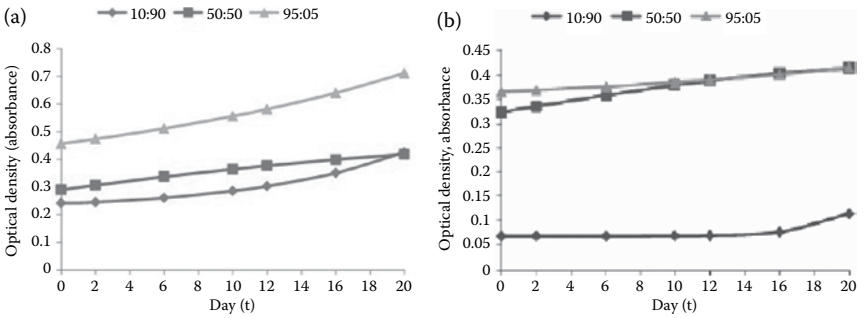

FIGURE 3.4
(a and b) OD of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time.

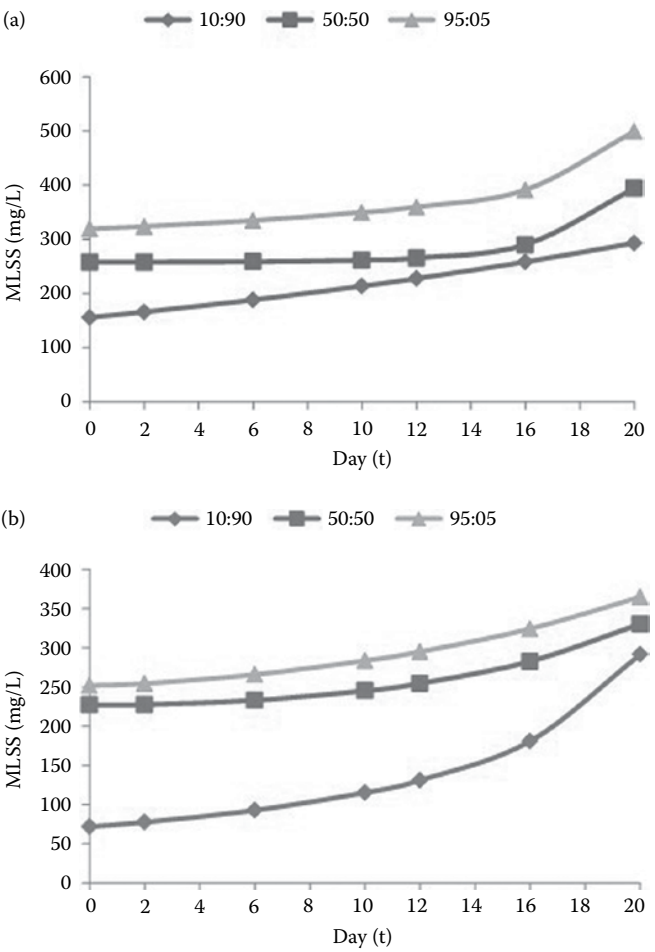

FIGURE 3.5
(a and b) MLSS of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time.

when conducting the experiment. Ratio 95:05 for both strains shows a higher starting point and continuously leading the growth, compared to the ratio 10:90. It shows that the ratio is suitable for the strains in producing high biomass thus producing high lipid content (Figures 3.6a and b and 3.7a and b).

3.4.6 Lipid

The gravimetric method has been used to obtain lipid mass production. From the findings obtained, comparison between cell dry weight and lipid has been made. Figure 3.8a shows cell dry weight versus lipid of *Chlorella*

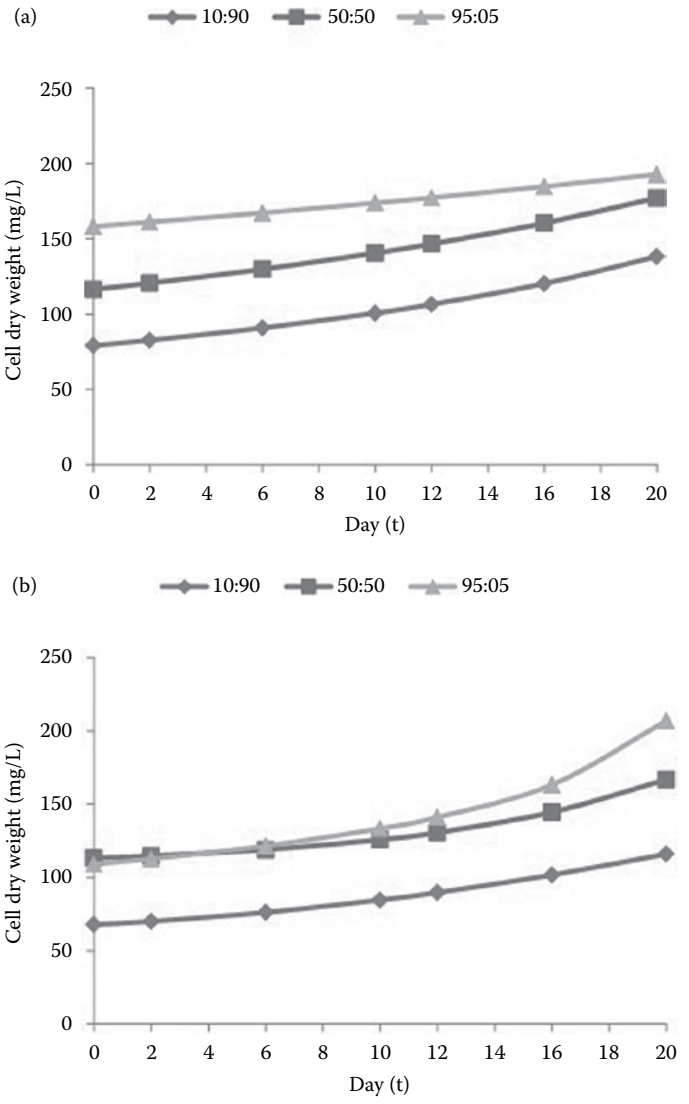

FIGURE 3.6
(a and b) Cell dry weight of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time.

vulgaris. It shows that at a ratio of 95:05, the lipid production is higher than the other ratio. At day 20, the cell dry weight obtained was 193 mg/L and the lipid was 56 mg/L compared to ratio 10:90, the cell dry weight obtained is 139 mg/L, a lipid is 40 mg/L and ratio 5:50 shows cell dry weight 177 and 51 mg/L for lipid production.

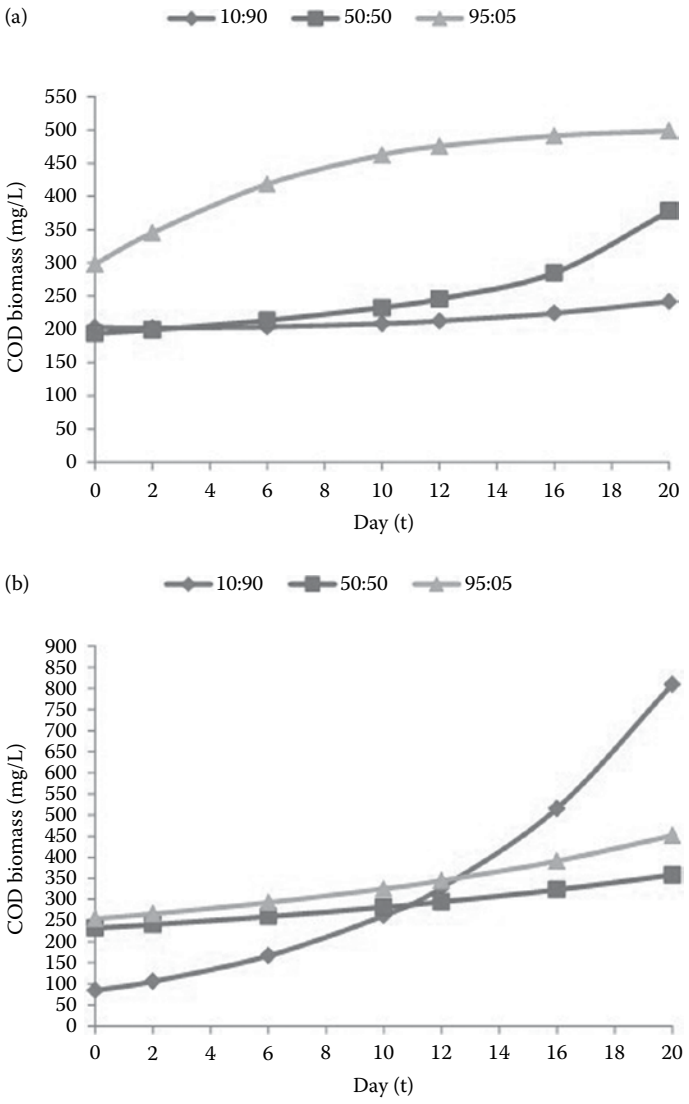

FIGURE 3.7
(a and b) COD biomass of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time.

Figure 3.8b indicates cell dry weight versus lipid of *Chlorella pyrenoidosa*. It shows at ratio 95:05, the lipid production is higher than the other ratio. At day 20, the cell dry weight obtained was 207 mg/L, and the lipid was 60 mg/L compared to ratio 10:90. The cell dry weight obtained is 116 and 34 mg/L for lipid production. To sum up, it was concluded that *Chlorella pyrenoidosa* produces higher lipid content compared to *Chlorella vulgaris* at 95:05 ratio.

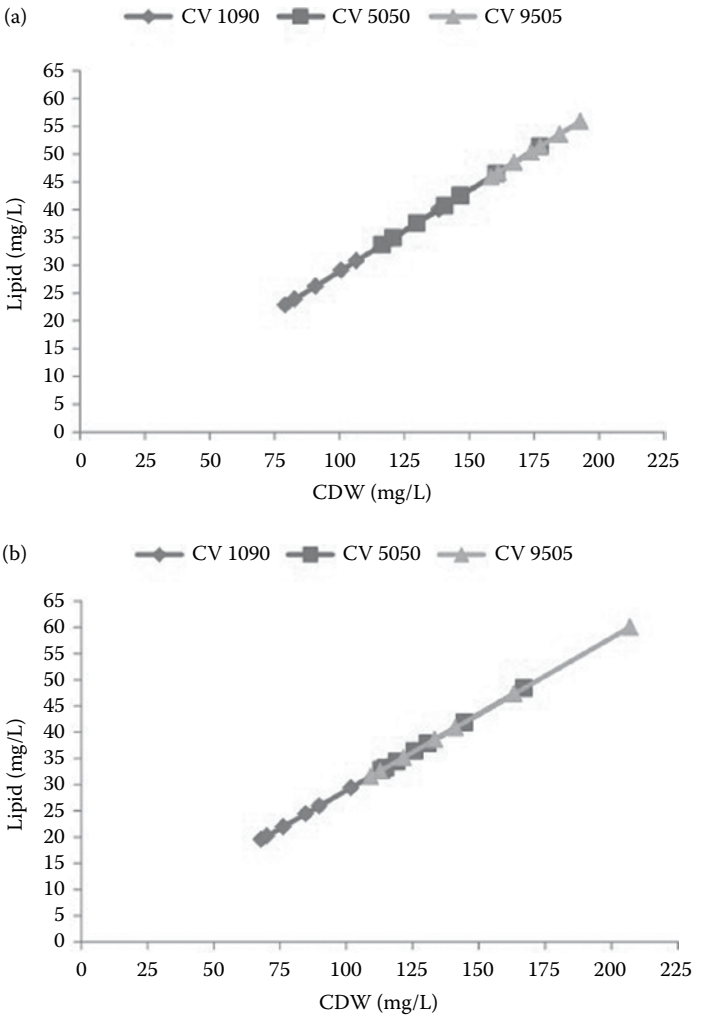

FIGURE 3.8
(a and b) Cell dry weight of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus lipid.

3.4.7 Ratio MLVSS/MLSS

Ratio of MLVSS/MLSS is another way to know the production of lipid other than quantifying the lipid. It can be used as an early prediction of lipid production for each ratio and strains before the lipid test was done. The analysis above shows that for both strains, ratio 10:90 will produce higher lipid content rather than the 95:05 and 50:50 ratios. It shows that both strains have higher MLVSS value over MLSS. The result can vary when the quantifying lipid test was done. (Figure 3.9a and b, MLVSS/MLSS of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time).

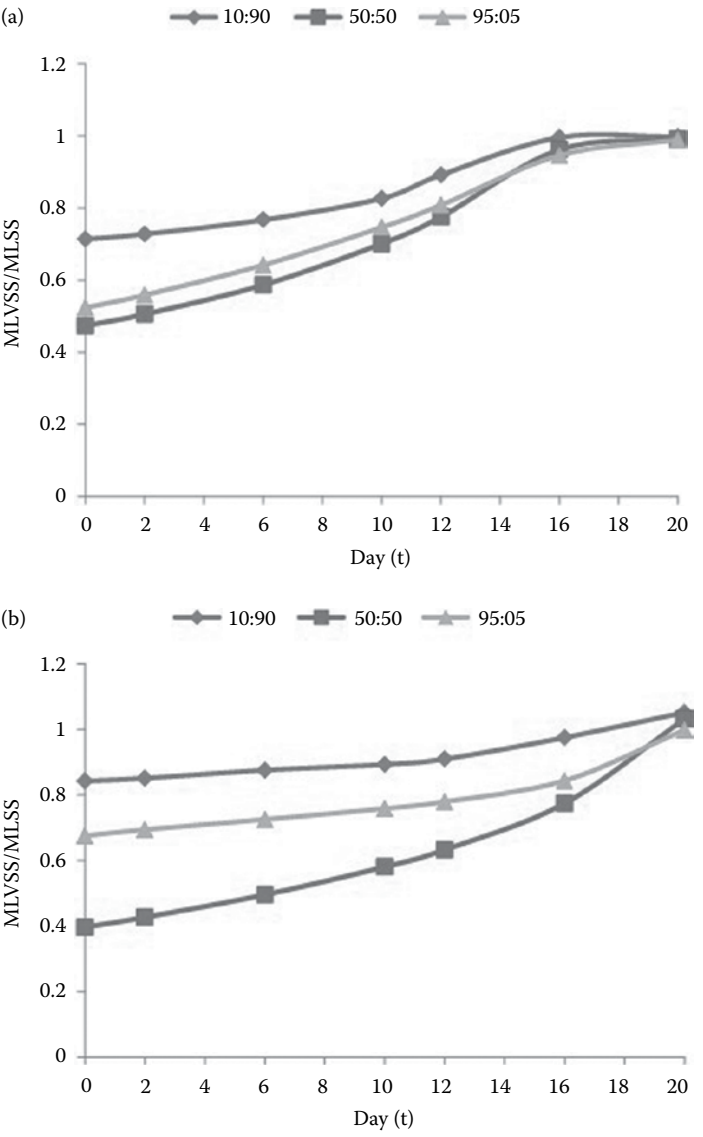

FIGURE 3.9
(a and b) MLVSS/MLSS of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time.

3.4.8 Chlorophyll-a, Lipid, and Biomass

Biomass, lipid, and chlorophyll-a are used in analyzing the best strain with best nutrient ratio to determine optimization of lipid content. High biomass results in high production of lipid. Figure lipid shows ratio 95:05 produces high lipid over time. The analysis shows that the lipid production is directly

proportional to the biomass generated. Chlorophyll relates to the process of photosynthesis for plants. Ratio 50:50 shows higher absorbance of chlorophyll-a for both strains. Ratio 95:05 for both strains shows lower absorbance of chlorophyll-a. It shows that for ratio 95:05 the chlorophyll used is not for survival but when there is depletion in nutrients, the chlorophyll acts as nutrient for lipid production.

Insert (Figure 3.10a and b Chlorophyll-a of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time)

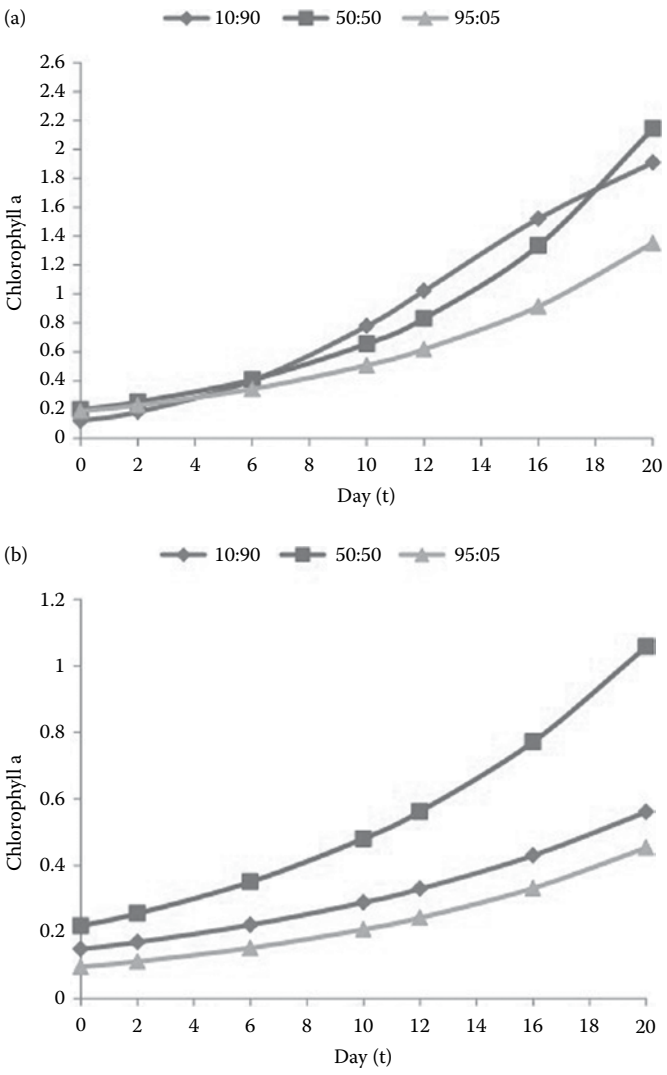

FIGURE 3.10
(a and b) Chlorophyll-a of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time.

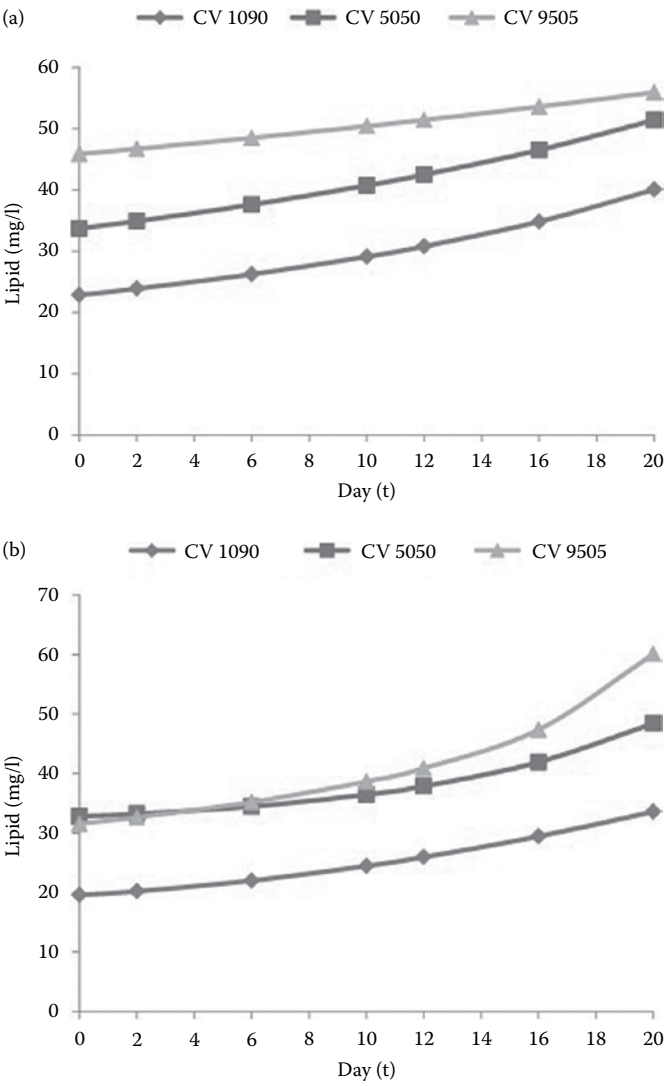

FIGURE 3.11
(a and b) Lipid of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time.

Insert (Figure 3.11a and b lipid of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time)

3.4.9 Lipid Productivity

The lipid content was determined, and the lipid production was calculated based on the results. For both strains, all ratios show increment over time.

Ratio 95:05 leads the production with the highest productivity from day 0 till day 20. As discussed earlier, the lipid content depends on the nitrogen limitation.

Insert (Figure 3.12a and b lipid productivity of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time)

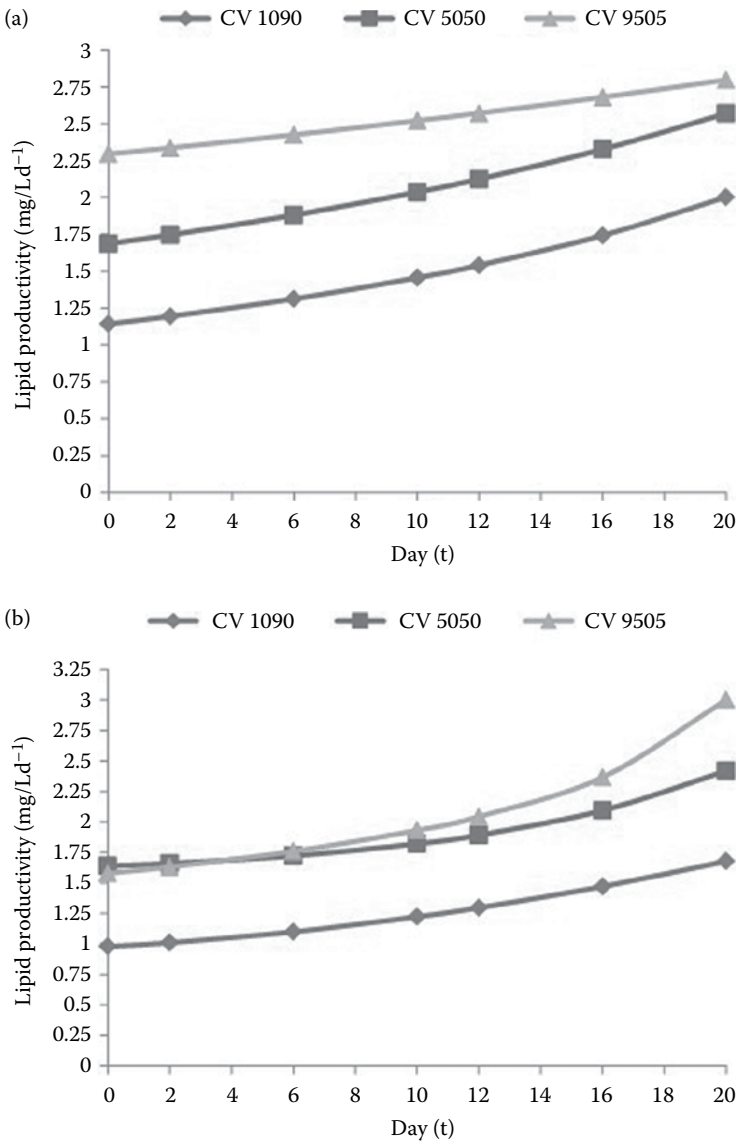

FIGURE 3.12
(a and b) Lipid productivity of *Chlorella vulgaris* and *Chlorella pyrenoidosa* versus time.

3.5 Conclusion

Based on the results obtained from the experiments and analysis, some conclusions can be drawn:

1. *Chlorella pyrenoidosa* was found to be dominant, and the POME condition is favorable for *Chlorella pyrenoidosa*.
2. *Chlorella vulgaris* can adapt and grow well in a POME environment.
3. Optimization of lipid content is best at ratio 95:05 and *Chlorella pyrenoidosa* produces higher lipid content compared to *Chlorella vulgaris*.

3.6 Recommendations

Some recommendations are suggested for future research in this area:

1. Lipid content can be optimized by varying photo light duration from the best strain and nutrient ratio obtained from this study.
2. Lipid production in heterotrophic condition can be investigated using the optimized nutrient ratio.

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